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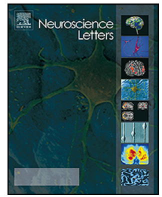

Research article

Monocarboxylate transporter-dependent mechanism confers resistance to oxygen- and glucose-deprivation injury in astrocyte-neuron co-cultures

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HIGHLIGHTS

- 8 h OGD significantly increases cell death in primary neuronal cultures.
- OGD up-regulates MCT4 expression in primary astrocyte cultures.
- Neuronal cell death in co-cultures is increased by exposure to MCTs-specific siRNA under OGD.
- Exogenous lactate in the extracellular medium can protect neuronal cultures from OGD.

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ABSTRACT

Hypoxic and low-glucose stressors contribute to neuronal death in many brain diseases. Astrocytes are anatomically well-positioned to shield neurons from hypoxic injury. During hypoxia/ischemia, lactate released from astrocytes is taken up by neurons and stored for energy. This process is mediated by monocarboxylate transporters (MCTs) in the central nervous system. In the present study, we investigated the ability of astrocytes to protect neurons from oxygen- and glucose-deprivation (OGD) injury via an MCT-dependent mechanism in vitro. Primary cultures of neurons, astrocytes, and astrocytes–neurons derived from rat hippocampus were subjected to OGD, MCT inhibition with small interfering (si)RNA. Cell survival and expression of MCT4, MCT2, glial fibrillary acidic protein, and neuronal nuclear antigen were evaluated. OGD significantly increased cell death in neuronal cultures and up-regulated MCT4 expression in astrocyte cultures, but no increased cell death was observed in neuron–astrocyte co-cultures or astrocyte cultures. However, neuronal cell death in co-cultures was increased by exposure to MCT4- or MCT2-specific siRNA, and this effect was attenuated by the addition of lactate into the extracellular medium of neuronal cultures prior to OGD. These findings demonstrate that resistance to OGD injury in astrocyte–neuron co-cultures occurs via an MCT-dependent mechanism.

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1. Introduction

Astrocytes are involved in the physical structuring of the brain. They are the most abundant glial cells in the brain that are closely associated with neuronal synapses [1]. Glial cells are also involved in providing neurotrophic signals to neurons required for their survival, proliferation, and differentiation [2]. In addition, reciprocal interactions between glia and neurons are essential for many critical functions in brain health and disease. Glial cells play pivotal roles in neuronal development, activity, plasticity, and recovery

Abbreviations: MTCs, monocarboxylate transporters; OGD, oxygen and glucose deprivation; GFAP, glial fibrillary acidic protein; NeuN, neuronal nuclear antigen; FBS, fetal bovine serum; DMEM/F12, Dulbecco's Modified Eagle's Medium/F12; RT-PCR, real-time polymerase chain reaction; SD, standard deviation; HIF-1<alpha>, hypoxia-inducible factor<alpha>.

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from injury [3]. The idea that astrocytes have active roles in the modulation of neuronal activity and synaptic neurotransmission is now widely accepted [4]. Lactate released from astrocytes via glycogenolysis and glycolysis is taken up by neurons and used for energy [5]. Monocarboxylate transporters (MCTs), which are abundantly expressed in neurons and astrocytes, play an important role in this process [6]. MCTs belong to the SLC16 gene family, which comprises 14 members. MCT1–4 are proton symporters that mediate the transmembrane transport of lactate, pyruvate, and ketone bodies [7]; MCT2 is expressed primarily in neurons in the brain, while MCT4 is expressed almost exclusively in astrocytes [8]. During hypoxia/ischemia, lactate released from astrocytes is taken up by neurons and stored for energy via up-regulation of MCT4 expression [9]. Glial fibrillary acidic protein (GFAP) is an astrocyte-differentiation marker and is considered to be an important element in astrocyte differentiation and in the reactive response to central nervous system injury [10].

In the present study, we investigated the ability of astrocytes to protect neurons from oxygen- and glucose-deprivation (OGD) injury via an MCT-dependent mechanism in vitro. Primary neuronal, astrocyte, and astrocyte–neuron co-cultures derived from rat hippocampus were subjected to OGD, and MCT4, MCT2, GFAP and neuronal nuclear antigen (NeuN) expression levels were evaluated.

2. Materials and methods

2.1. Rat primary astrocyte–neuron co-cultures

All procedures were approved by the Animal Care and Use Committee of LanZhou University (Lanzhou, China) and followed the National Guidelines for Animal Experimentation. Hippocampuses were obtained from 18-day Sprague–Dawley rat embryos ($n = 7$), using a modification of a previously-described method [11]. The cells were plated on culture flasks or glass coverslips in a six-well plate at around $1–2 \times 10^5$ cells/cm^2 and maintained in Neurobasal-A growth medium without fetal bovine serum (FBS) at 37 °C in 5% CO_2/95% air (balanced nitrogen and 85% humidity) in an incubator (i.e., normoxic conditions). The culture medium was replaced completely 4–6 h after plating and half the medium was then changed every 3–4 days thereafter. On the 4th day after plating, 1.5 mM leucine–leucine methyl ester was added to the medium to deplete microglia in the astrocyte–neuron co-cultures. The cells were used for experiments 2 weeks after plating.

2.2. Rat primary neuronal cultures

Pure neuronal cultures were derived from the same embryonic hippocampal cell suspensions as the above co-cultures. Astrocyte growth was inhibited by adding cytosine arabinoside (10 μM) 72 h after plating and by decreasing the serum content in the medium. The cells were used for experiments 2 weeks after plating.

2.3. Rat primary astrocyte cultures

Hippocampal astrocytes were obtained from postnatal day 1 Sprague–Dawley rats ($n = 10$) and maintained in Dulbecco's Modified Eagle's Medium/F-12 (1:1: Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% FBS and a mixture of 100 U/ml penicillin and 100 μg/ml streptomycin. The culture medium was replaced completely 1 h after plating to eliminate fibroblasts, and half the medium was then changed every 3–4 days thereafter. On the 4th day after plating, 1.5 mM leucine–leucine methyl ester was added to the medium to deplete microglia. Cells were detached with trypsin and replated at least twice before use. The cells were used approximately 3 weeks after plating.

2.4. OGD model and cell treatment

The three kinds of primary cultures were subjected to hypoxia by OGD, according to an established protocol [12]. Briefly, OGD was produced in an anaerobic chamber (Coy Laboratory Products Inc., Grass Lake, MI, USA). Oxygen levels were monitored continuously with a gas analyzer (Coy Laboratory Products Inc.). On the day of OGD treatment, cells were provided with fresh culture medium without glucose and serum and placed in the anaerobic chamber flushed with 1% O_2/5% CO_2/95% N_2 at 37 °C. After 8 h, the culture medium was replaced and cells were maintained under normoxic conditions for 48 h.

2.5. Incubation of neuronal cultures with L-lactate

L-Lactate (Sigma–Aldrich, St. Louis, MO, USA) was added into the medium of neuronal cultures at a concentration of 5.5 mmol/l, as described previously [13], followed by subsequent experiments.

2.6. Detection of cell death by immunofluorescence

The survival of cells on coverslips was assessed using a live/dead cell viability kit (Molecular Probes, Invitrogen, Singapore). Ethidium homodimer-1 stains the DNA in dead cells red when exposed to light at 528 nm, while calcein-AM penetrates live cells and fluoresces green when exposed to light at 494 nm. Ten fields on each coverslip were photographed using the filter sets (blue: 488/515 nm; green: 514/550 nm) for each fluorescent label. The percentages of live and dead cells were calculated in relation to the total (live + dead) cell count. Cells on the coverslips were then fixed with 4% paraformaldehyde, rinsed, and treated with the nuclear counterstain DAPI to detect apoptotic cells. DAPI-stained cells (10 per coverslip) were captured, and apoptotic cells were identified by their bright, condensed, or multi-fragmented nuclei.

2.7. RNA interference

Predesigned, silencing, small interfering RNAs (siRNAs) were obtained from Ambion (Austin, TX, USA) and nucleofected into cultured cells using a Nucleofector device (Amaxa Inc. Gaithersburg, MD, USA). Approximately, 2 × 106 primary cells were resuspended in 100 μl nucleofector solution containing Lipofectamine 2000 transfection reagent (#11668-027, Invitrogen) and Opti-MEM I reduced serum medium (#31985-070, Gibco, rand Island, NY, USA). Astrocyte cultures were incubated with MCT4 (Slc16a3)-specific (#4390771) or negative control siRNA (#4390843), and neuronal cultures were incubated with MCT2 (Slc16a7)-specific (#AM16708) or negative control siRNA (#AM4611), according to the manufacturer's instructions. The mixtures were transferred to an electroporation cuvette with a 2 mm gap (Eppendorf, Hamburg, Germany) and nucleofection was performed in the Nucleofector device using the U-031 program. Transfected cells were retrieved from the cuvette with 500 μl warm, reduced-serum medium and allowed to recover for at least 5 min at 37 °C. The cells were used for subsequent experiments 72 h after the beginning of transfection.

2.8. Real-time polymerase chain reaction

MCT4 mRNA levels in astrocyte cultures and MCT2 mRNA levels in neuronal cultures were detected by real-time polymerase chain reaction (RT-PCR). Quantitative RT-PCR was performed for 45 cycles at 95 °C for 15 s and 60 °C for 1 min using an IQ5 Multicolor real-time PCR detection system (Biorad, München, Germany) for a 1/10 volume of the RT reaction mixture and TaqMan Gene Expression Assays (Applied Biosystems, Foster City, CA, USA), with rat β-actin as a loading control. The primer sequences were as follows:

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